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Original Article

Mycobacterium avium and *Mycobacterium abscessus* complex target distinct cystic fibrosis patient subpopulations

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Abstract

Background: Clinical observations suggest that *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MABSC) may affect cystic fibrosis (CF) patients with different characteristics and risk factors, but this has never been demonstrated within a single prospective cohort.
Methods: We studied 50 MABSC-positive and 23 MAC-positive patients from a French prevalence study of non-tuberculous mycobacteria (NTM) in CF. Risk factors specifically associated with MABSC and MAC were analyzed by nested case–control studies, with two NTM-negative controls matched by age, sex and center for each case.
Results: MAC-positive patients were significantly older than MABSC-positive patients (mean [SD] age, 23.1 [10.2] vs 17.4 [8.3] years, $p=0.013$), and were also older at CF diagnosis (mean [SD] age, 12.9 [16.1] vs 3.1 [7.7] years, $p=0.015$); they tended to be less frequent of the ΔF508/ΔF508 genotype (33.3 vs 61.1%, $p=0.17$) and to use pancreatic extracts less frequently (82.4 vs 97.6%, $p=0.07$). Risk factors identified by multivariate analysis were: i) in the MAC case–control study, an older age at CF diagnosis ($p=0.004$); ii) in the MABSC case–control study, at least one course of intravenous antibiotics ($p=0.01$) and more frequent isolation of *Aspergillus* ($p=0.03$).
Conclusions: MAC affects adult patients with a mild form of CF, whereas MABSC affects younger patients with more severe CF and more frequent intravenous antimicrobial treatment.

Keywords: *Mycobacterium avium* complex; *Mycobacterium abscessus* complex; Non-tuberculous mycobacteria; Cystic fibrosis; Mycobacterial lung disease

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1. Introduction

Since the 1990s, an increasing number of studies have reported the isolation of non-tuberculous mycobacteria (NTM) from the respiratory tract of patients with cystic fibrosis (CF) [1–7]. NTM prevalence surveys worldwide show that *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MABSC) account for over 95% of cases of NTM lung disease in CF patients [3,4,6,7]. MAC, a member of the slowly growing mycobacteria subgroup, is the most prevalent among such cases in North America [3]. MABSC, a member of the rapidly growing mycobacteria subgroup, however, is more prevalent than MAC in Western Europe [6,7] and Israel [4].

MAC respiratory infection seems to have only a small effect, if any, on the health of CF patients. Indeed, the large North American multicenter study of NTM in CF showed that NTM-positive patients (mostly MAC-positive) had better pulmonary function than NTM-negative controls [3]. MABSC-positive patients, by contrast, frequently have severe, sometimes fatal, lung disease [5,8–11]. Our own and other studies have suggested that these differences between MAC-positive and MABSC-positive patients are linked to a particularly high degree of virulence of MABSC in CF patients [12–14]. However, some findings suggest that there are differences between the CF populations affected by MAC and MABSC, which may substantially affect the severity of mycobacterial lung disease in these patients. Unlike MAC, MABSC infects young children [7,15] and may be associated with allergic bronchopulmonary aspergillosis and systemic steroid therapy [16]. In the North American multicenter study, *Pseudomonas aeruginosa* was less frequent in NTM-positive patients (mostly MAC-positive, see above) than in NTM-negative controls [3].

The question of whether MABSC and MAC "target" different subpopulations of patients has never been addressed in multicenter studies, due to the small numbers of cases, recruitment bias (for example, study populations in which patients are mostly children or mostly adults) or the retrospective nature of the study. We recently carried out a large survey of NTM in French CF patients, which included almost 1600 subjects [6]. MABSC and MAC were the most common NTM, and were the only NTM associated with being positive for American Thoracic Society (ATS) bacteriological criteria for NTM lung disease. Here, we compared the characteristics of the MABSC-positive and MAC-positive patients from this cross-sectional survey, and further investigated the risk factors associated with infection by each organism in two nested case–control studies.

2. Patients and methods

2.1. The French NTM prevalence study

The 2004 French NTM prevalence study included 1582 CF patients (mean age, 18.9 years; extremes, 4 months to 82 years) from 41 of the 49 French CF centers (17 adult centers, 20 pediatric centers and four mixed adult and pediatric centers) between January 1st 2004 and December 31st 2004 [6]. This survey included all patients capable of providing at least three sputum samples (or other respiratory specimens) for NTM analysis, with no age limit. Samples were decontaminated with the two-step *N*-acetyl-L-cysteine-NaOH-oxalic acid method as previously described [6,7].

2.2. The French cystic fibrosis registry

Since 1992, individual medical and social data for CF patients attending the 49 national CF centers in France have been obtained annually using a standardized questionnaire. The chief medical officer from each center filled in patient data sheets during annual check-ups and data were entered into a national database. All patients, or their parents if they were children, gave their informed consent, and an internal review board from the Observatoire National de la Mucoviscidose approved the study.

2.3. Comparison of characteristics of MABSC-positive and MAC-positive patients

All MABSC-positive and MAC-positive patients (at least one sample positive for MABSC or MAC, respectively) from the French NTM prevalence study were included in this analysis. Two MABSC-positive patients who were also positive for other NTM species (only one positive sample found for each case) were not excluded from the MABSC group. Demographic, clinical and laboratory data were obtained from the national CF registry. The chi-square test (or Fisher's exact test if necessary) was used for comparisons of qualitative variables, and Student's test for quantitative variables (STATA software Version 9, StataCorp LP). Statistical significance was accepted for $p < 0.05$.

2.4. Nested case–control studies of risk factors associated with MABSC and MAC

The case–control studies included patients who were positive for MAC only or for MABSC only, with two NTM-negative controls matched by age (controls born within ±2, 5 and 10 years of the birth date for cases aged 0–15, 16–29 and ≥30, respectively), sex and center for each case. When more than two eligible controls were found for one case, the controls were selected at random. Only cases and controls with data available from the national registry for both 2003 and 2004 were included in the case–control study.

The risk factors studied included items related to the diagnosis of CF (age at diagnosis, *CFTR* genotype) and the following parameters, collected the year before the patients were recruited into the French prevalence study: body mass index (BMI) and forced expiratory volume in one second (FEV_1) at the annual check-up (both measured at the same time during the last consultation or last hospitalization during the year considered); insulin-dependent diabetes mellitus; sputum microbiology (*P. aeruginosa*, methicillin-resistant *Staphylococcus aureus*,

Table 1
Comparative analysis of MABSC-positive and MAC-positive patients from the French NTM survey.

Characteristics	MABSC-positive patients (n=50)	MAC-positive patients (n=23)	p-value
Sex ratio	0.79	0.53	0.64
Mean age (SD) at inclusion, years	17.4 (8.3)	23.1 (10.2)	0.013
Mean age (SD) at CF diagnosis, years	3.1 (7.7)	12.9 (16.1)	0.015
ΔF508/ΔF508 [a]	61.1% (n=36)	33.3% (n=12)	0.11
Mean (SD) BMI, kg/m^2	18.2 (3.3) (n=36)	19.1 (3.3) (n=17)	0.37
Mean (SD) FEV1, % predicted	53.6 (19.4) (n=30)	62.2 (24.7) (n=15)	0.20
Mean (SD) sweat chloride, mM/L	110 (30.3) (n=28)	100.3 (32.8) (n=14)	0.35
Use of pancreatic extracts [a]	97.6% (n=42)	82.4% (n=17)	0.07
Mean (SD) no. of days of hospitalization [b]	14.7 (44.4) (n=33)	7.9 (21.0) (n=17)	0.55
P. aeruginosa [a,c]	57.5% (n=40)	50.0% (n=16)	0.77
MSSA [a,c]	50.0% (n=40)	62.5% (n=16)	0.55
MRSA [a,c]	20.0% (n=40)	12.5% (n=16)	0.70
Aspergillus sp. [a,c]	37.5% (n=40)	31.3% (n=16)	0.76

Abbreviations: NA, not available; SD, standard deviation; MABSC, *M. abscessus* complex; MAC, *M. avium* complex; BMI, body mass index; FEV1, forced expiratory volume in one second.
[a] Frequency; (): total no. evaluated.
[b] The year preceding inclusion in the study.
[c] At least one positive sample in the year before inclusion.

Aspergillus sp.) throughout the year; treated allergic bronchopulmonary aspergillosis (ABPA); number of inpatient hospitalizations; number of intravenous antibiotic courses; long-term (≥3 months) use of inhaled therapies (antibiotics, rhDnase, steroids and bronchodilators); azithromycin maintenance treatment; long-term (≥3 months) use of systemic steroids and non-steroid anti-inflammatory (NSAI) drugs.

Cases and controls were compared using the likelihood ratio test. MABSC- and MAC-associated risk factors were analyzed by estimating univariate and multivariate odds ratios (ORs) with 95% confidence intervals (95% CI), using conditional logistic regression [17]. P-values were assessed by the likelihood ratio test (univariate analysis) and the Wald test (multivariate analysis). All factors with a p-value ≤ 0.2 in the univariate analysis were included in the multivariate analysis. Exact tests were used when necessary (SAS/STAT software, version 9.1, SAS Institute Inc., Cary, NC). We considered associations with a p-value < 0.05 to be statistically significant.

3. Results

3.1. Characteristics of MAC-positive and MABSC-positive patients

Of the 1582 patients included in the multicenter French survey of NTM in CF, 50 had at least one sample that was found positive for MABSC (MABSC-positive patients), and 23 for MAC (MAC-positive patients); 80% (40/50) of MABSC-positive patients and 73.9% (17/23) of MAC-positive patients met ATS bacteriological criteria for NTM lung disease [18].

As shown in Table 1, MAC-positive patients were significantly older at NTM diagnosis (mean [SD] age, 23.1 [10.2] vs 17.4 [8.3] years, p=0.013) and at CF diagnosis (mean [SD] age, 12.9 [16.1] vs 3.1 [7.7] years, p=0.015) than MABSC-positive patients. MAC-positive patients also showed a trend towards being less frequent of the ΔF508/ΔF508 genotype (33.3 vs 61.1%, p=0.17) and for less frequent use of pancreatic extracts (82.4 vs 97.6%, p=0.07) than MABSC-positive patients.

We analyzed the distribution of MAC-positive and MABSC-positive patients as a function of age at CF diagnosis. The distribution patterns differed considerably between the two subgroups (Fig. 1). Nearly 90% of MABSC-positive patients were diagnosed for CF before 10 years of age and only about 5% after 19 years. By contrast, less than 60% of MAC-positive patients were diagnosed for CF before 10 years of age; and one third were diagnosed after 19 years of age and one quarter after 30.

3.2. MAC case–control study

Ten of the 23 MAC-positive patients were excluded from the nested MAC case–control study (medical history not available, 6 patients; 2003 data not available, 4 patients). We thus included 13 MAC cases, all fulfilling ATS bacteriological criteria for NTM lung disease [18], and 26 matched controls. Cases and controls had the same sex ratio (M/F, 0.3) and comparable ages (mean [CI 95%] age, 22.8 [18.6–27.1] and 23.3 [19.1–28.3] years, respectively).

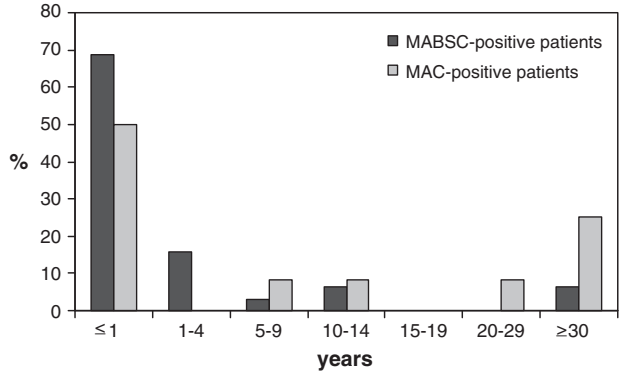

Fig. 1. Distribution of MAC-positive and MABSC-positive patients as 325 a function of age at CF diagnosis.

The only factor positively significantly associated with MAC by univariate analysis was older age at CF diagnosis (Table 2). A negative association was found for the use of inhaled bronchodilators in the year before inclusion in the study (table). Among the factors tested by multivariate analysis (p-value ≤ 0.2 in the univariate analysis), only greater age at CF diagnosis was found to be significantly associated with MAC (Table 2). This prompted us to compare the circumstances of CF diagnosis in MAC cases and their controls: intestinal symptoms (which are associated with early diagnosis) were significantly less likely in MAC cases (Supplemental file, Table 1).

3.3. MABSC case–control study

Twenty of the 50 MABSC-positive patients were excluded from the nested MABSC case–control study (medical history not available, 9 patients; 2003 data not available, 8 patients; positivity for other NTM, 2 patients; discordant sex data, 1 patient). We thus included 30 MABSC cases, all fulfilling ATS bacteriological criteria for NTM lung disease [18], and 60 controls. Cases and controls had the same sex ratio (M/F, 1.0) and comparable ages (mean [CI 95%] age, 17.3 [14.7–19.9] and 17.4 [15.6–19.2] years, respectively). In univariate analysis, MABSC showed a positive association with administration of at least one course of i.v. antibiotics in the preceding year (Table 3). None of the other risk factors studied were found to be significantly associated with MABSC. Among the factors tested by multivariate analysis (p-value ≤ 0.2 in the univariate analysis), only two showed a positive association with MABSC: i) at least one i.v. antibiotic course in the year before inclusion in the study; ii) isolation of *Aspergillus* the year before inclusion in the study (Table 3). Two other factors showed a negative association: i) FEV1 < 40% predicted the year before inclusion in the study; ii) inhaled steroids taken the year before inclusion in the study (Table 3).

4. Discussion

In this study, we used the large, multicenter French survey of NTM in CF [6], which allowed, for the first time, comparisons between MAC-positive and MABSC-positive patients from the same CF population in the same year. Recruitment biases were minimized because the French NTM study included all patients capable of providing three (or more) sputum samples for NTM analysis, with no age limit and no selection according to the clinical status of the patients. Moreover, information was collected independently from the national CF registry and only data from this register were used. For the analysis of risk factors, appropriate control groups were obtained by matching cases and controls for age, gender and center. Age and gender need to be taken into account because they are significantly associated with NTM in CF patients [6,7]. However, our preliminary findings using data from the French CF registry showed substantial differences in the therapeutic management of patients between participating centers (e.g., frequency of use of aerosols or long-term azithromycin). Also, there may be significant differences concerning the environmental exposure to NTM between patients attending centers in different geographical regions [19]. Thus we considered it important to exclude any potential 'center effect', particularly as this issue has been overlooked in previous studies.

Our results showed a clear tendency of MAC to infect adults with mild CF. With a mean age of 23.1 years, MAC-positive patients were significantly older than MABSC-positive patients (17.4 years). MAC was only very rarely isolated from patients younger than 10 years of age; nearly 75% of MAC-positive patients were 16 years old or older. This group of patients could also be clearly distinguished by their older age at CF diagnosis (mean age 12.9 years vs 3.1 years for MABSC-positive patients), a finding that has never been reported previously to our knowledge. One third of MAC-positive patients were diagnosed for CF only after 19 years of age. In multivariate analysis, the diagnosis of CF at an older age was the only risk factor associated with isolation of MAC. This finding is consistent with MAC preferentially infecting a subpopulation of patients with a less severe form of CF, which may consequently be detected at a later age. The link between MAC and a less severe form of CF is also consistent with the less frequent use of pancreatic extracts in MAC-positive patients.

It remains unclear why a less severe form of CF disease and/or late diagnosis of CF disease may predispose patients to infection by MAC. It is possible that antibiotic treatments may prevent the development of MAC in the respiratory tract ("beneficial" effect of antibiotics), but not MABSC (see below). It is also possible that late diagnosis of CF results in delayed and less stringent application of the appropriate hygiene and control measures [20]. MAC is present in a wide variety of environmental settings, including water, soil and dust [19]. A recent study found high levels of MAC rRNA sequences in showerhead biofilms [21]. Recommendations on the home environment (regular de-scaling and disinfection of lavatories and bathroom fittings, etc.) (http://vaincrelamuco.org) may thus help to reduce the risk of acquiring MAC.

Unlike MAC, MABSC tended to infect children and adolescents with a more severe form of CF (more frequent ΔF508/ΔF508 genotype and use of pancreatic extracts, lower FEV1 values, longer hospital stays the year before inclusion), although differences were not significant. In multivariate analysis,

Table 2
Analysis of risk factors associated with MAC.

Risk factors	Cases (n=13)	Controls (n=26)	Univariate OR [CI 95%]	p-value	Multivariate OR [CI 95%] [a]	p-value
Mean age at CF diagnosis, years [CI 95%] [b]	**13.3 [4.0–22.6] n=13**	**3.3 [0.8–5.5] n=25**	**1.2 [1.0–1.4]**	**0.004**	**1.2 [1.0–1.4]**	**0.004**
ΔF508/ΔF508 [c]	**3/10 (30)**	**13/24 (54.17)**	**0.3 [0.08–1.5]**	**0.1**		**NS**
BMI<20 [c]	**7/13 (53.85)**	**19/25 (76.0)**	**0.4 [0.09–1.8]**	**0.2**		**NS**
FEV1<40% predicted [c]	2/13 (15.38)	8/25 (32.0)	0.4 [0.08–2.3]	0.3		NI
ID diabetes mellitus [c]	0/13 (0)	1/26 (3.9)	[d]	–		NI
Pseudomonas aeruginosa [c,e]	6/13 (46.15)	16/26 (61.54)	0.5 [0.1–2.1]	0.3		NI
MRSA [c,e]	3/13 (23.08)	4/26 (15.38)	1.6 [0.3–8.4]	0.6		NI
Aspergillus [c,e]	**8/13 (61.54)**	**11/26 (42.31)**	**3.1 [0.6–16.9]**	**0.2**		**NS**
Treated ABPA [c]	3/13 (23.08)	4/26 (15.38)	2 [0.3–14.2]	0.5		NI
At least one hospital stay [c]	3/11 (27.27)	9/26 (34.62)	0.6 [0.1–3.5]	0.6		NI
At least one i.v. antibiotic course [c]	7/13 (53.85)	16/26 (61.54)	0.7 [0.2–2.9]	0.6		NI
Inhaled antibiotics [c,f]	**3/13 (23.08)**	**11/26 (42.31)**	**0.4 [0.07–1.9]**	**0.2**		**NS**
Inhaled rhDnase [c,f]	5/13 (38.46)	12/26 (46.15)	0.7 [0.2–3.1]	0.6		NI
Inhaled steroids [c,f]	**2/13 (15.38)**	**9/26 (34.62)**	**0.4 [0.07–1.9]**	**0.2**		**NS**
Inhaled bronchodilators [c,f]	**3/13 (23.08)**	**15/26 (57.69)**	**0.1 [0.01–0.9]**	**0.01**		**NS**
Long-term azithromycin [c,f]	**2/13 (15.38)**	**11/25 (44.0)**	**0.2 [0–1.1]**	**0.07**		**NS**
Oral steroids [c,f]	1/13 (7.69)	1/22 (4.55)	1.4 [0.08–23.6]	0.8		NI
Oral NSAI [c,f]	0/13 (0)	0/24 (0)	– [d]	–		NI

Abbreviations: CF, cystic fibrosis; 95% CI, 95% confidence interval; OR, odds ratio; BMI, body mass index; FEV1, forced expiratory volume in one second; ID, insulin-dependent; MRSA, methicillin-resistant *Staphylococcus aureus*; ABPA, allergic bronchopulmonary aspergillosis; i.v., intravenous; NSAI, non-steroid anti-inflammatory drugs; NI, variables not included; NS, not significant.

[a] Only variables for which univariate analysis showed a p-value lower than 0.2 (bold) were tested by multivariate analysis.
[b] Linearity was checked: a one unit increase corresponds to a one year increase.
[c] No./total no. evaluated (%).
[d] The Log-likelihood ratio test was not applicable.
[e] At least one positive sputum sample.
[f] At least three consecutive months.

Table 3
Analysis of risk factors associated with MABSC.

Risk factors	Cases (n=30)	Controls (n=60)	Univariate OR [CI 95%]	p-value	Multivariate OR [CI 95%] [a]	p-value
Mean age at CF diagnosis, years [CI 95%] [b]	2.6 [0.12–4.99] n=29	2.7 [1.41–4.01] n=56	1.0 [0.9–1.1]	1.0		NI
ΔF508/ΔF508 [c]	16/27 (59.26)	34/53 (64.15)	0.7 [0.3–1.4]	0.3		NI
BMI<20 [c]	19/28 (67.86)	45/59 (76.27)	0.6 [0.2–2.1]	0.4		NI
FEV1<40% predicted [c]	**2/26 (7.69)**	**10/56 (17.86)**	**0.4 [0.09–2.0]**	**0.2**	**0.04 [0.002–1.03]**	**0.05**
ID diabetes mellitus [c]	**3/27 (11.11)**	**2/59 (3.39)**	**3 [0.5–18.0]**	**0.2**		**NS**
Pseudomonas aeruginosa [c,d]	18/30 (60)	34/58 (58.6)	1.1 [0.5–2.6]	0.9		NI
MRSA [c,d]	5/30 (16.67)	8/58 (13.79)	1.2 [0.4–3.6]	0.8		NI
Aspergillus [c,d]	**14/30 (46.67)**	**18/58 (31.03)**	**2.2 [0.8–5.8]**	**0.1**	**6.0 [1.2–29.4]**	**0.03**
Treated ABPA [c]	**6/27 (22.22)**	**6/59 (10.17)**	**2.4 [0.6–8.7]**	**0.2**		**NS**
At least one hospital stay [c]	6/25 (24)	21/51 (41.18)	0.5 [0.1–1.8]	0.3		NI
At least one i.v. antibiotic course [c]	**23/29 (79.31)**	**34/57 (59.65)**	**2.8 [1.0–8.1]**	**0.04**	**13.7 [1.7–109.8]**	**0.01**
Inhaled antibiotics [c,e]	15/30 (50.0)	35/58 (60.34)	0.7 [0.3–1.6]	0.4		NI
Inhaled rhDnase [c,e]	18/30 (60.0)	32/58 (55.17)	1.3 [0.5–3.7]	0.6		NI
Inhaled steroids [c,e]	**8/30 (26.67)**	**26/58 (44.83)**	**0.4 [0.1–1.2]**	**0.08**	**0.06 [0.006–0.7]**	**0.03**
Inhaled bronchodilators [c,e]	10/30 (33.33)	25/58 (43.10)	0.6 [0.2–1.7]	0.4		NI
Long-term azithromycin [c,e]	10/28 (35.71)	23/58 (39.66)	0.9 [0.3–2.3]	0.8		NI
Oral steroids [c,e]	1/27 (3.70)	2/54 (3.7)	0.6 [0.05–7]	0.7		NI
Oral NSAI [c,e]	2/30 (6.67)	3/57 (5.26)	1.2 [0.2–7.0]	0.9		NI

Abbreviations: CF, cystic fibrosis; 95% CI, 95% confidence interval; OR, odds ratio; BMI, body mass index; FEV1, forced expiratory volume in one second; ID, insulin-dependent; MRSA, methicillin-resistant *Staphylococcus aureus*; ABPA, allergic bronchopulmonary aspergillosis; i.v., intravenous; NSAI, non-steroid anti-inflammatory drugs.

[a] Only variables for which univariate analysis showed a p-value lower than 0.2 (bold) were tested by multivariate analysis.
[b] Linearity was checked: a one unit increase corresponds to a one year increase.
[c] No./total no. evaluated (%).
[d] At least one positive sputum sample.
[e] At least three consecutive months.

despite a trend to better FEV1 values than their controls, MABSC cases were more likely to have received intravenous antimicrobial treatment. The increased use of intravenous antimicrobial therapy may provide an ideal terrain for MABSC infection. MABSC is one of the most resistant mycobacterial species, and expresses natural resistance to most intravenous antibiotics commonly used in cases of CF [22]. The more frequent isolation of the antibiotic-resistant *Aspergillus* spp. from MABSC-positive patients supports this hypothesis. As for some other emerging CF pathogens [23,24], MABSC infections may thus be a side effect of intravenous antibiotic treatments. This is consistent with the results of the Israeli multicenter study, which also found a link between NTM – mostly MABSC and *M. simiae* – and the increased likelihood that patients received intravenous antimicrobial treatment [4]. We cannot however exclude the possibility that the more frequent i.v. antimicrobial treatment is not a cause but rather a consequence of MABSC infection: such treatment may be prescribed for infectious symptoms months or even years before the identification of MABSC.

We did not find a significant positive association between the presence of MABSC or MAC in sputum and the use of the other "new" therapeutic approaches to CF such as inhaled therapies or long-term azithromycin [25,26]. We detected a negative association between MAC and bronchodilator aerosols (univariate analysis only), probably due to the fact that MAC infects subjects with milder forms of CF. Our results do not however exclude the possibility that positive associations exist between MABSC or MAC infection and new therapeutic approaches; indeed, the acquisition of NTM may be associated with exposure to such treatments months, or even years, earlier. Indeed, the main limitation of our study is that, like all other published studies, any such association could have been missed. Although the therapy administered to any one patient does not generally change substantially from one year to the next, particularly when the clinical status is stable, bias of this type cannot be excluded.

Consistent with the study by Levy et al. [4], we did not identify ABPA as a significant risk factor for MABSC. This contrasts with an earlier Israeli study by Mussaffi et al., which found a significant association between NTM infections – mostly MABSC infections – and both ABPA and systemic steroid therapy [16]. These discrepancies may result from differences in the definitions used or – most probably – from an inadequate control group in the study by Mussaffi et al. However, the absence of an association between MABSC and ABPA (treated or not) is consistent with *Aspergillus* spp. only being a marker of exposure to broad-spectrum antibiotics. The negative association in multivariate analysis between inhaled steroid therapy and MABSC in the case–control study is also consistent with these conclusions.

Our results clearly show that not only does the apparent severity of an NTM infection in a CF patient result from the severity of the infection itself, but that it also depends on the severity of CF. In the case of MAC infections, which arise principally in adults in relatively good health, mycobacterial infection may be missed. In the case of MABSC infections, which arise frequently in young children with poor respiratory function and general state, the effect of the mycobacterial infection can be overestimated. Diagnostic ATS criteria for NTM lung disease are often difficult to apply to CF patients, due to overlapping symptoms and radiographic changes attributable to the CF disease itself [18]. Our data show that it may be useful to identify diagnostic and prognostic criteria that are specifically associated with MABSC and MAC in CF patients, as has previously been attempted for radiological data [27]. This would help decisions about whether or not to treat, currently the major issue facing clinicians in the management of CF patients positive for NTM.

In conclusion, we show that MABSC and MAC target CF patient populations with different, and even opposite, profiles, and are associated with independent and different risk factors. Thus, if we are to progress in the diagnosis, treatment and prevention of NTM lung infections in CF patients, clinical and epidemiological studies will be needed in which NTM are no longer considered as a homogeneous entity.

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